

## **Annexin A2 facilitates endocytic trafficking of antisense oligonucleotides**

### **SUPPLEMENTARY DATA**

#### **Supplemental materials**

##### **Antibodies**

ANXA2 (610069), S100A10 (610071), and GRP78 (610978) antibodies were from BD Biosciences. LAMP1 (9091S), ALIX (2171S), and Rab7 (9367S) antibodies were from Cell Signaling Technology. ANXA3 (PA5-27884), ANXA4 (PA5-27871) antibodies were from Life Technologies. GADPH (sc-25778) antibody was from Santa Cruz Biotechnology. Anti-rabbit (170-6515) and anti-mouse (170-6516) secondary antibodies conjugated to HRP were from Bio-Rad. Rab11 (ab3612) antibody and Anti-rabbit secondary antibodies conjugated to AF488 (ab150077) and AF647 (ab150079) as well as anti-mouse secondary antibodies conjugated to AF488 (ab150113) and AF647 (ab150115) were purchased from Abcam.

##### **siRNAs**

The siRNA targeting human *ANXA2* (sc-270151) was from Santa Cruz Biotechnologies. The siRNAs targeting mouse *ANXA2* (159942 and 161612), human *S100A10* (12231 and 12324), human *ANXA3* (S1386 and S1387), and human *ANXA4* (147064 and 147063) and human Rab11 (S16702 and S17648) were from Life Technologies.

## **Primer probe sets for qRT-PCR**

ANXA2:

Forward: 5'- GATGAGGTCACCATTGTCAACATT-3'

Reverse: 5'- GGCGAAGGCAATATCCTGTCT-3'

Probe: 5'- TGACCAACCGCAGCAATGCACA-3'

Drosha:

Forward: 5'- CAAGCTCTGTCCGTATCGATCA-3'

Reverse: 5'- TGGACGATAATCGGAAAAGTAATCA-3'

Probe: 5'-CTGGATCGTGAACAGTTCAACCCCGAT-3'

Malat1:

Forward: 5'-GCTTGGCTTCTTCTGGACTCA-3'

Reverse: 5'-TCGCGAGCTTCACCATGA-3'

Probe: 5'- CGCCACTTGTCCGCTTCACACTCC-3'

Mouse SRB1:

Forward: 5'-TGACAACGACACCGTGTCCT-3'

Reverse: 5'-ATGCGACTTGTCAGGCTGG-3'

Probe: 5'-CGTGGAGAACCGCAGCCTCCA-3'

Mouse Malat1:

Forward: 5'-GTAGGTTAAGTTGACGGCCGTTA-3'

Reverse: 5'-ATCTTCCCTGTTTCCAACATCATG-3'

Probe: 5'-AAAAATCCTTCGACTGGCGCATGTACG-3'

Primer probe sets for ANXA3 (Hs00192983\_m1), ANXA4 (Hs00154040\_m1), S100A10 (Hs00751478\_s), Rab11a (HS00366449\_g1) and Rab11b (HS00188448\_m1) were from Life Technologies.

## Oligonucleotides

IONIS ID 116847 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C\* T\*C\*T\* G\*G\*A\* T\*T\*T\* G\*A- 3'. The underlined nucleotides are 2'-O-MOE modified, and the \* indicates phosphorothioate backbone.

IONIS ID 446654 is of the same sequence and chemistry as IONIS ID 116847 but is labeled at 5' end with Cy3.

IONIS ID 395254 targets human Malat1: 5'-G\*G\*C\* A\*T\*A\* T\*G\*C\* A\*G\*A\* T\*A\*A\* T\*G\*T\* T\*C- 3'. The underlined nucleotides are 2'-O-MOE modified, and the \* indicates phosphorothioate backbone

IONIS ID 399479 targets mouse Malat1: 5'-C\*G\*G\*T\*G\*C\*A\*A\*G\*G\*C\*T\*T\*A\*G\*G\*A\*A\*T\*T\*- 3'. The underlined nucleotides are 2'-O-MOE modified, and the \* indicates phosphorothioate backbone.

IONIS ID 25690 targets *Drosha*: 5'-A\*T\*C\* C\*C\*T\* T\*T\*C\* T\*T\*C\* C\*G\*C\* A\*T\*G-3'. The underlined nucleotides are 2'-O-MOE modified, and the \* indicates phosphorothioate backbone.

IONIS ID 205382 targets mouse *SRB1*: 5'-C\*A\*C\*C\*T\*C\*T\*G\*C\*C\*A\*C\*G\*T\*A\*C\*A\*G\*T\*G-3'. The underlined nucleotides are 2'-O-MOE modified, and the \* indicates phosphorothioate backbone.

IONIS ID 626823 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C\* T\*C\*T\* G\*G\*A\* T\*T\*T\* G\*A-3'. The underlined bold letters indicate 2'-cEt modified nucleotides, and the \* indicates phosphorothioate backbone, labeled at 5' end with Fluorescein.

IONIS ID 626825 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C\* T\*C\*T\* G\*G\*A\* T\*T\*T\* G\*A-3'. The underlined letters indicate 2'-fluoro-modified nucleotides, and the \* indicates phosphorothioate backbone. The oligonucleotide was labeled at the 5' end with Cy3.

IONIS ID 391857 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C\* T\*C\*T\* G\*G\*A\* T\*T\*T\* G\*A-3'. The underlined nucleotides are LNA modified, and the \* indicates phosphorothioate backbone. The oligonucleotide was labeled at the 5' end with fluorescein.

IONIS ID 364617 targets *PTEN*: 5'-CTGCTAGCCTCTGGATTGA-3'. The underlined are 2'-MOE modified nucleotides. The backbone is phosphodiester.

IONIS ID 364617 targets *PTEN*: 5'- C\*T\*G\* C\*T\*A\* G\*C\*C\* T\*C\*T\* G\*G\*A\* T\*T\*T\* G\*A-3'. The underlined nucleotides are 2'-MOE modified, and \* indicates phosphorothioate backbone.

IONIS ID 386652 targets *PTEN*: 5'-biotin-C\*T\*G\* C\*T\*A\* G\*C\*C\* T\*C\*T\* G\*G\*A\* T\*T\*T\* G\*A-3'. The underlined are 2'-O-MOE modified nucleotides, and the \* indicates phosphorothioate backbone. Biotin is conjugated to the 5' end.

IONIS ID 730437 targets Malat1: 5'-Cy3-C\*C\*A\*G\*G\*C\*T\*G\*G\*T\*T\*A\*T\*G\*A\*C\*T\*C\*A\*G\*A-3'. The underlined are 2'-O-MOE modified nucleotides, and the \* indicates phosphorothioate backbone. The 3' end is conjugated with triantennary N-acetyl galactosamine, and the 5' end is modified with Cy3.

IONIS ID 841226 targeting Malat1: 5'-Cy3-CCAGGCTGGTTATGACTCAG-3'. The underlined are 2'-O-MOE modified nucleotides. The 3' end is conjugated with triantennary N-acetyl galactosamine and the 5' end with Cy3.

IONIS ID XL198 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C\* T\*C\*T\* G\*G\*A\* T\*T\*T\* G\*A-3'. The \* indicates phosphorothioate backbone. The oligonucleotide was labeled at the 5' end with Cy3.

IONIS ID 651101 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C-3'. The underlined are 2'-cEt modified nucleotides, and the \* indicates phosphorothioate backbone. The oligonucleotide is labeled at 5' end with Cy3.

IONIS ID 651102 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C\*T\*C-3'. The underlined are 2'-cEt modified nucleotides, and the \* indicates phosphorothioate backbone. The oligonucleotide is labeled at the 5' end with Cy3.

IONIS ID 651103 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A\* G\*C\*C\*T\*C\*T\*G-3'. The underlined are 2'-cEt modified nucleotides, and the \* indicates phosphorothioate backbone. The oligonucleotide is labeled at the 5' end with Cy3.

IONIS ID 651104 targets *PTEN*: 5'-C\*T\*G\* C\*T\*A GCCTCTGGA-3'. The underlined are 2'-cEt modified nucleotides, and the \* indicates phosphorothioate backbone. The oligonucleotide is labeled at the 5' end with Cy3.

## Supplemental figure legends

### Figure S1. Overexpression of ANXA2 does not alter ASO activity

**A)** Western analysis for ANXA2 protein levels in control HeLa cells or cells overexpress DKK-tagged ANXA2. Grp78 served as a loading control. **B)** Control or ANXA2 overexpressing cells were incubated with ASOs targeting either *Drosha* or *Malat1* RNAs for 16 hr and the RNA levels were determined using qRT-PCR.

### Figure S2. ANXA2 localization to LEs does not depend on ASO 2'-modification or sequence

**A)** Immunofluorescent staining of ANXA2 in HeLa cells treated with 2  $\mu$ M Cy3-labeled ASOs with different 2'-modifications for 8 hrs. Scale bars: 10  $\mu$ m. **B)** Immunofluorescence staining of ANXA2 and PS-ASO in HeLa cells treated with 2  $\mu$ M unlabeled PS-ASOs (IONIS ID 25690 and IONIS ID 395254). The ASOs were stained with a PS-ASO specific antibody. The co-localization is indicated by arrows. Scale bars: 10  $\mu$ m.

### Figure S3. ANXA2 and ASO co-localization does not depend on Cy3 fluorescent label

**A)** Immunofluorescence staining of ANXA2 (green) in HeLa cells that were either treated with 2  $\mu$ M Cy3-labeled PS-ASO (IONIS ID 446654, red) for 4 hrs (upper panels) or pre-incubated with 2  $\mu$ M unlabeled PS-ASO (IONIS ID 116847) for 4 hrs followed by addition of 2  $\mu$ M Cy3-labeled PS-ASO for an additional 4 hrs (lower panels). Scale bars, 10  $\mu$ m. Nuclei were labeled with DAPI. **B)** Quantification of loci per cell in that co-stain

with PS-ASO and ANXA2. The error bars represent standard deviations from results obtained from ~20 cells.

**Figure S4. ANXA3 and ANXA4 proteins localize to LEs in PS-ASO-treated cells**

Immunofluorescence staining for **A)** ANXA3 or **B)** ANXA4 in HeLa cells not treated with PS-ASO (left panels) and in cells incubated with 2  $\mu$ M PS-ASO (IONIS ID 446654) for 16 hrs (right panels). Cells were stained with Rab7 as a marker for LEs. The co-localization of the Annexin proteins with ASO and Rab7 is indicated by arrows in magnified regions. Scale bars, 10  $\mu$ m.

**Figure S5. Reduction of ANXA3 and ANXA4 did not affect ASO activity**

**A)** Western analysis for ANXA3 protein levels in A431 cells treated with a control siRNA (Luci-si) or two different ANXA3 siRNAs (ANXA3-si1 and ANXA3-si2). GAPDH served as a loading control. **B)** Control or ANXA3 reduced cells were incubated with ASOs targeting either Drosha or Malat1 RNAs for 16 hr and the RNA levels were determined using qRT-PCR. **C)** Western analysis for ANXA4 protein levels in A431 cells treated with either a control luciferase siRNA or two different ANXA4 siRNAs (ANXA4-si1 and ANXA4-si2). GAPDH served as a loading control. **D)** Control or ANXA4 reduced cells were incubated with ASOs targeting either Drosha or Malat1 RNAs for 16 hr and the RNA levels were determined using qRT-PCR. Error bars represent standard deviations from three independent experiments.

**Figure S6. Reduction of Rab11 did not affect ASO activity**

**A)** Immunofluorescence staining of Rab11 (green) in control cells or cells treated with ANXA2 siRNA that were incubated with 2  $\mu$ M Cy3-labeled PS-ASO (IONIS ID 446654, red) for 4 hrs. **B)** RT-PCR for Rab11a and Rab11b mRNA levels in HeLa cells treated with a control siRNA (Luci-si) or different Rab11 siRNAs (Rab11a-si and Rab11b-si). **C)** Control or Rab11 reduced cells were incubated with ASOs targeting either *Drosha* or *Malat1* RNAs for 16 hr and the RNA levels were determined using qRT-PCR. The error bars are standard deviations from three independent experiments.

**Figure S7. Blocking ANXA2 localization to LEs reduces ASO activity**

**A)** Late endosomal localization of ANXA2 is blocked by bafilomycin treatment. ANXA2 immunostaining was conducted on cells pre-treated with bafilomycin for 16 hrs then treated with 2  $\mu$ M PS-ASO for 8 hrs. Scale bars, 5  $\mu$ m. **B)** A431 cells were pre-treated with different concentrations of bafilomycin for 4 hrs, followed by incubation with PS-ASOs targeting *Drosha* or *Malat1* RNA. The levels of *Drosha* and *Malat1* RNAs were determined by qRT-PCR. The error bars indicate standard deviations from three independent experiments.

Figure S1

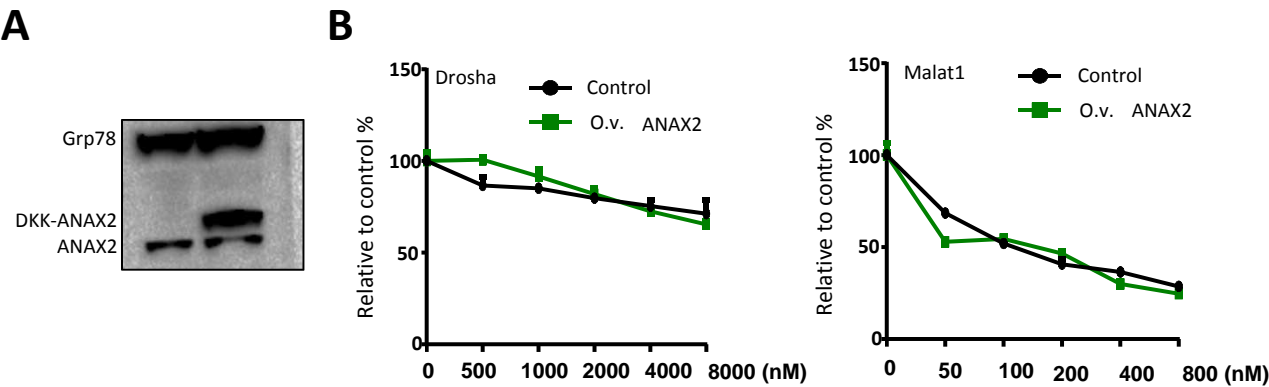
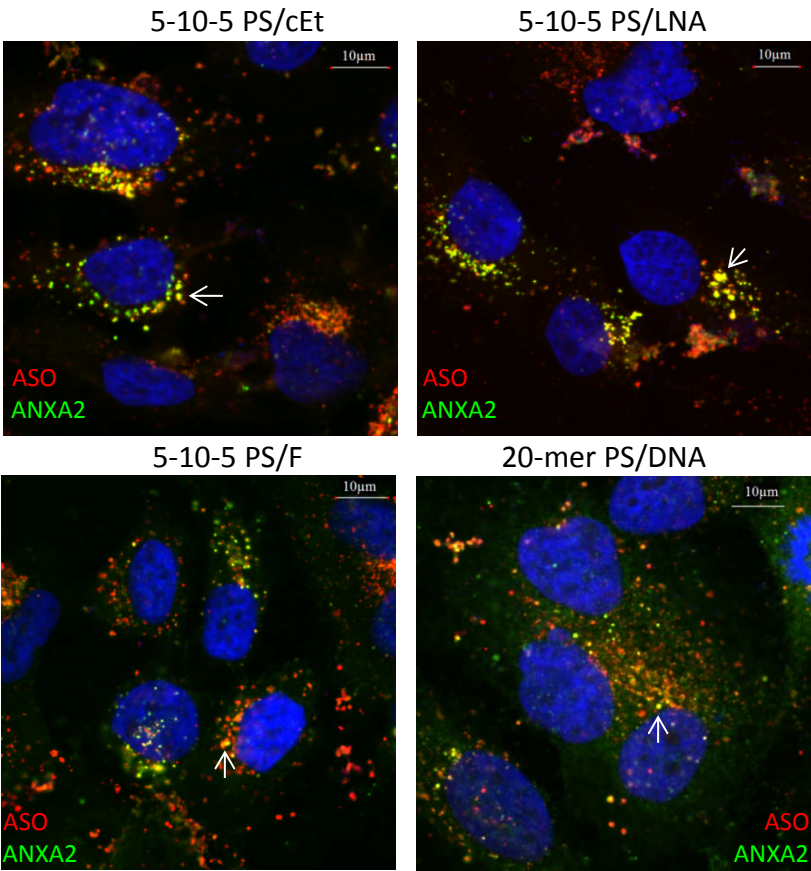


Figure S2

**A**



**B**

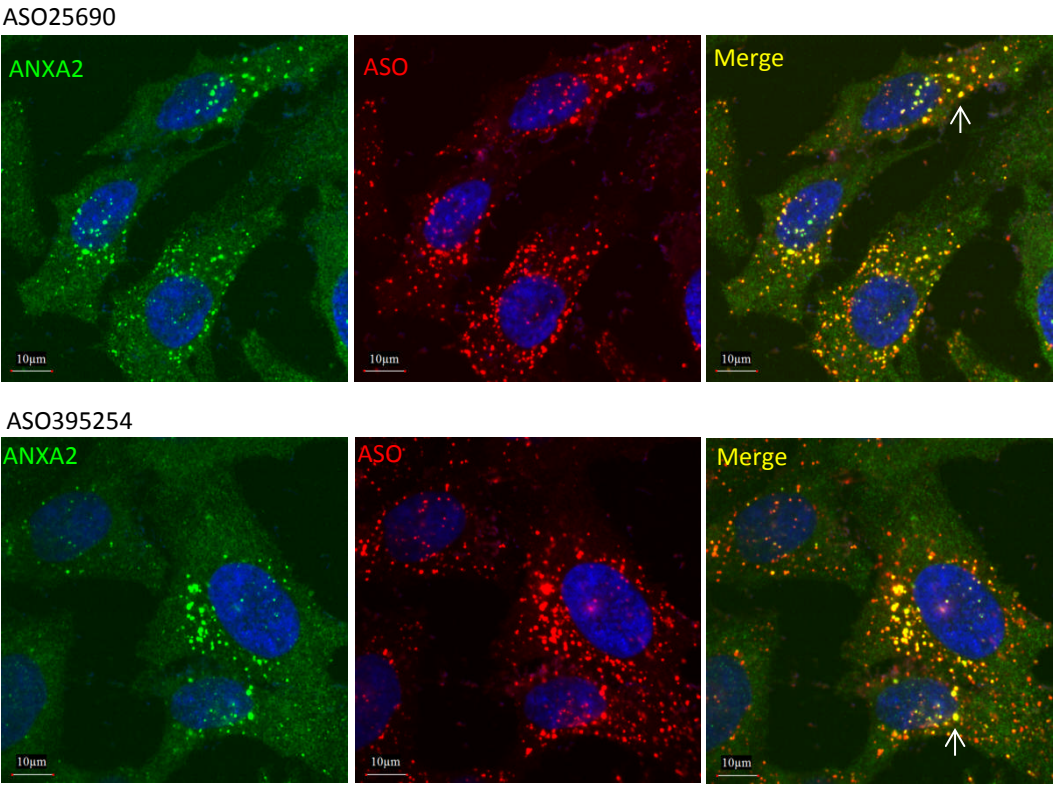


Figure S3

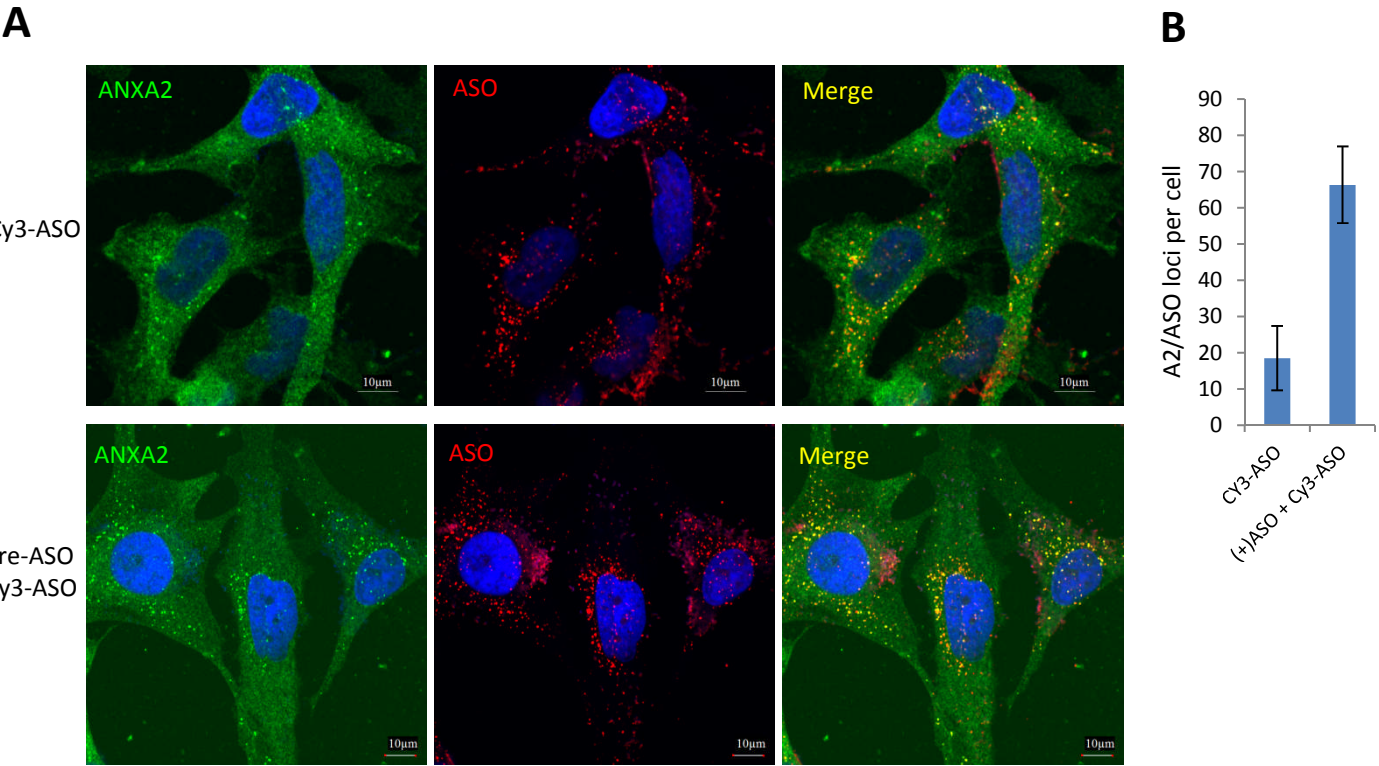
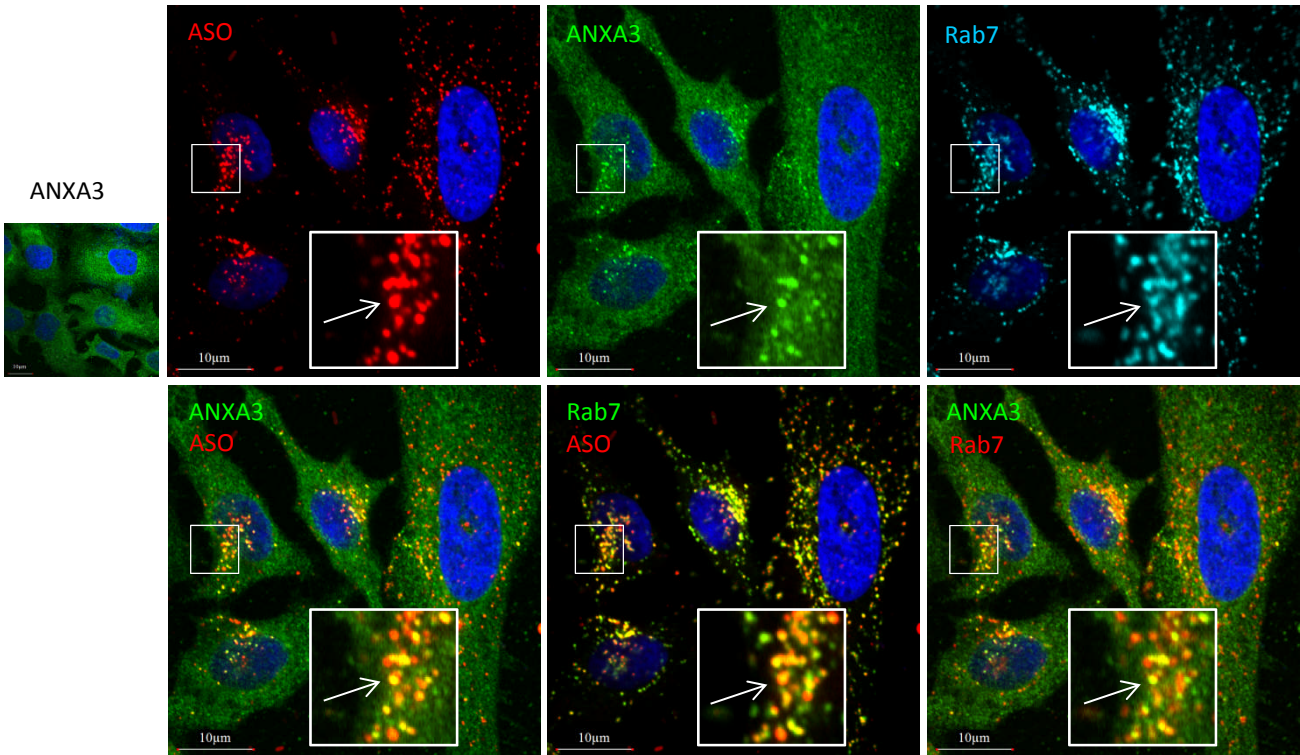


Figure S4

A



B

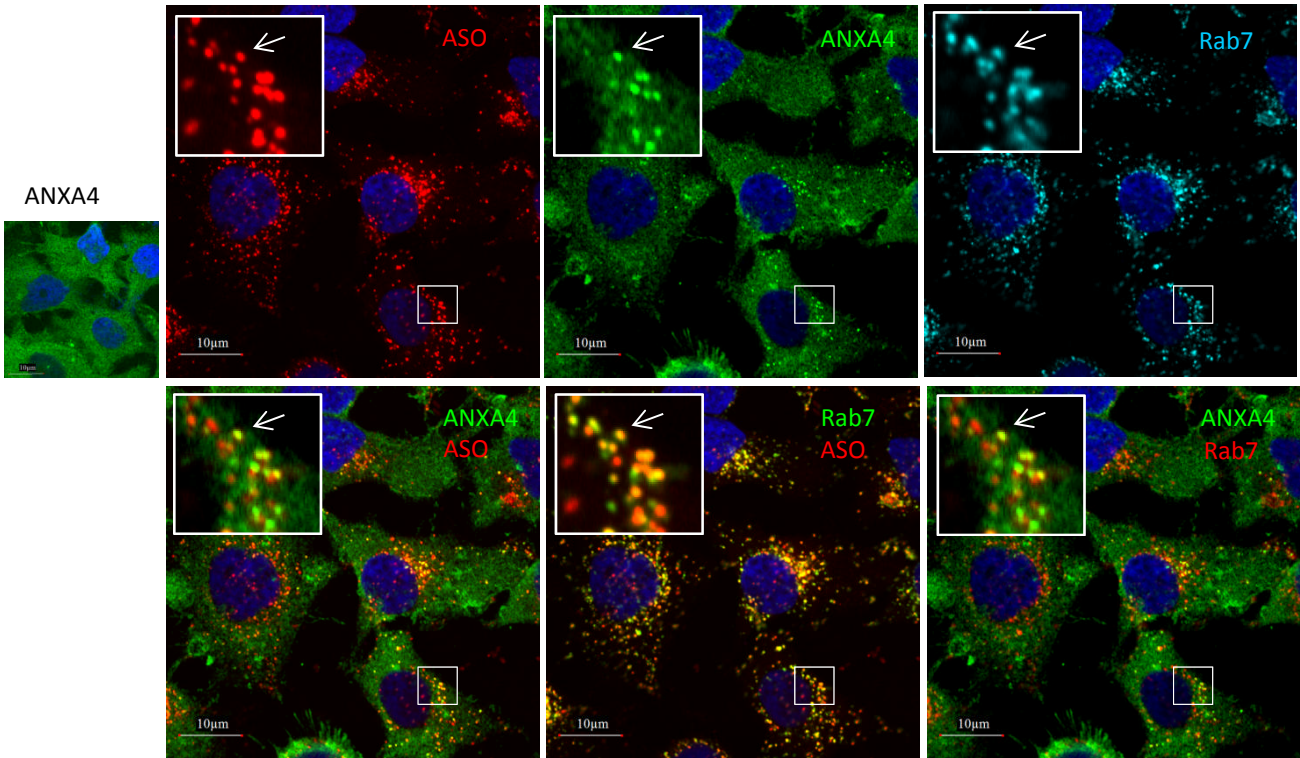


Figure S5

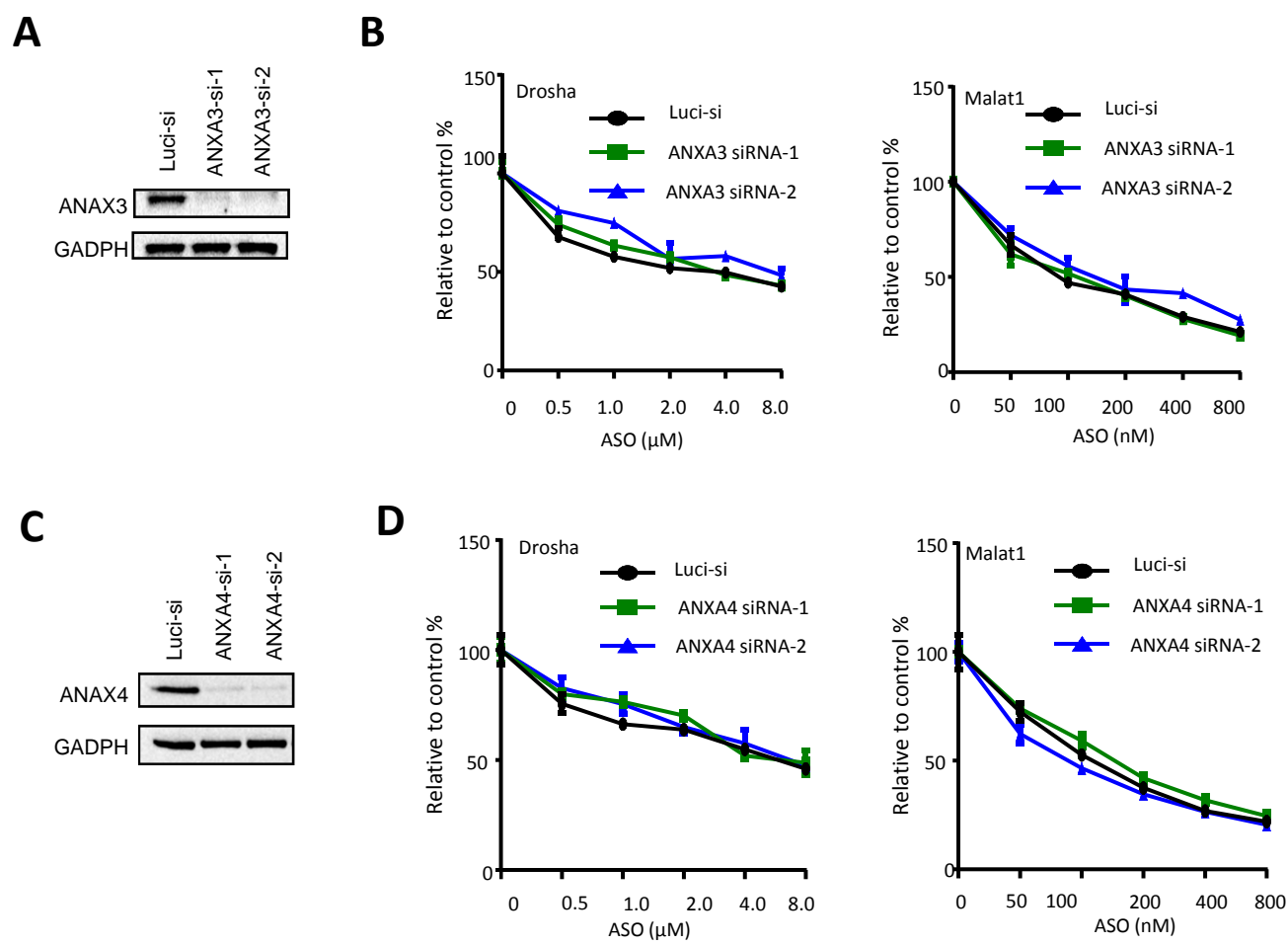
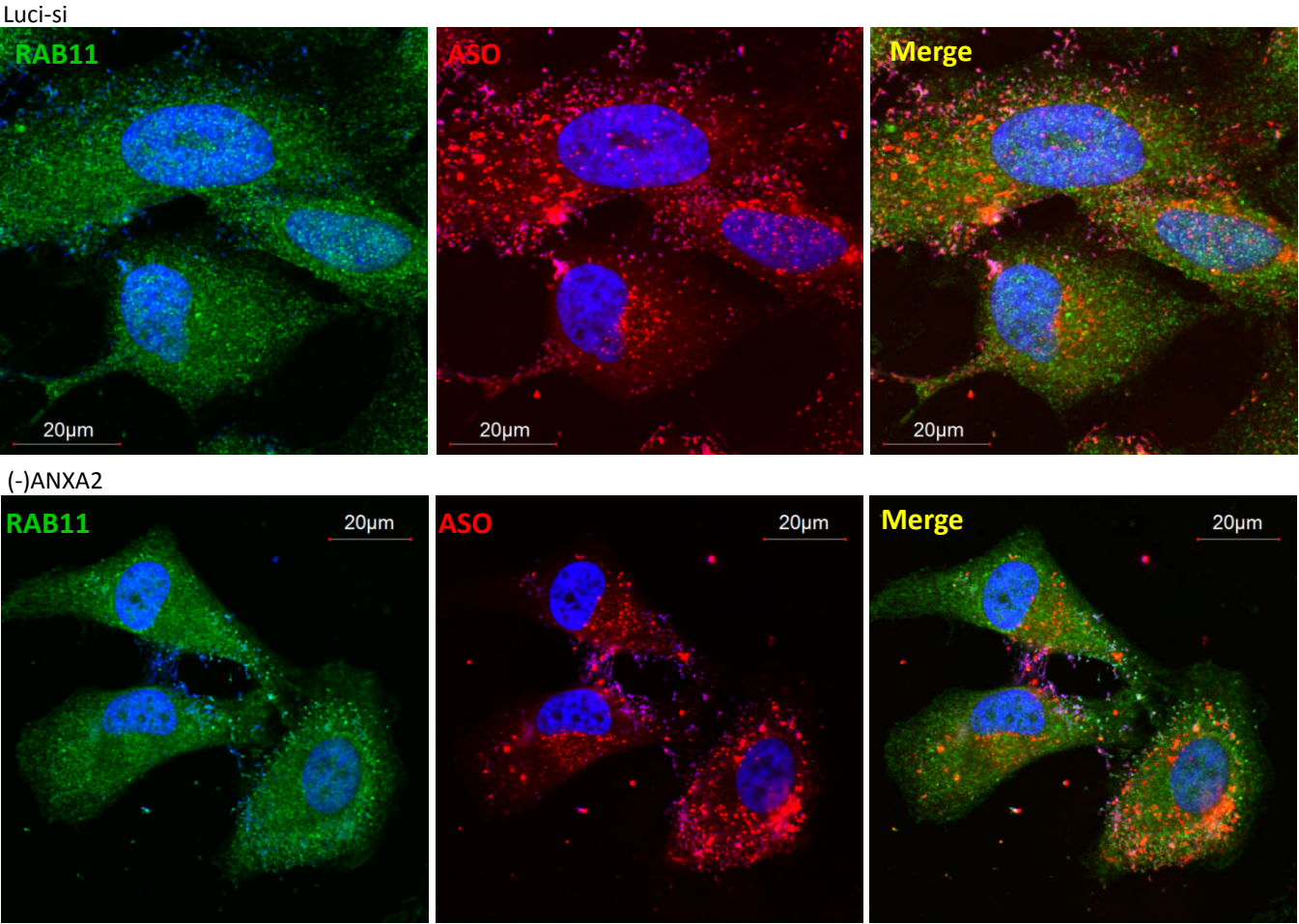
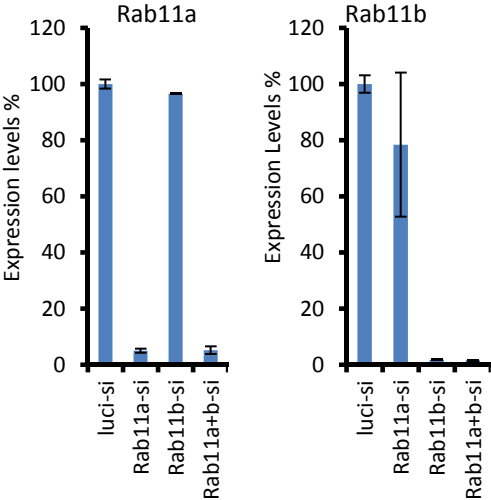


Figure S6

A



B



C

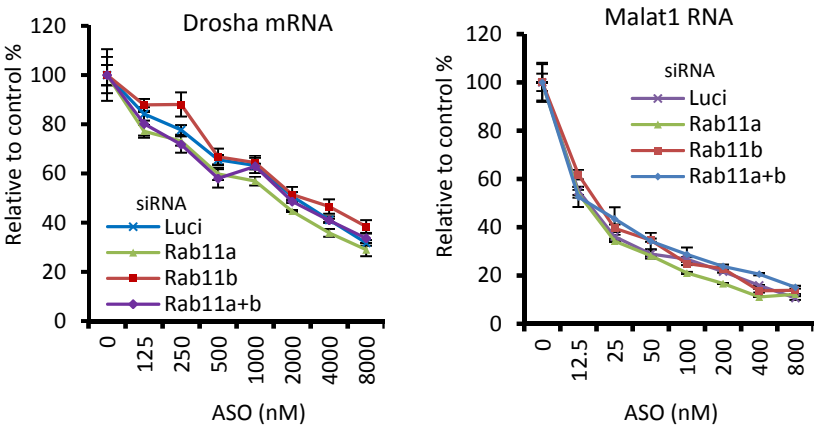


Figure S7

